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(71) Applicant and
(72) Inventor: HUYBRECHTS, Lucas [BE/BE]; Mina Telghuislaan 9, B-2550 Kontich (BE).

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(54) Title: TOOTH WHITENING PRODUCTS AND PROCEDURES

(57) Abstract: We have, surprisingly, discovered a "multi-stage" whitening procedure for teeth, in which several new and different types of whitening and stain weakening agents are used consecutively, together resulting in superior and improved whitening performance (up till full white teeth), and this under conditions of tremendously reduced levels of chemical exposure to teeth, as when compared with traditional whitening procedures based on the use of peroxides. In particular we have discovered that the tannase enzyme as well as dioxiranes effectively can whiten teeth; that the whitening performance of the papain enzyme can be enhanced substantially in the presence of selected co-ingredients; and that a pre-treatment with a laccase enzyme enhances the whitening performance of an oxidation chemical in a subsequent bleaching step. These new procedures provide both, improved and unmatched whitening performance, and this under conditions of improved and unmatched safety standards.

Tooth whitening products and procedures.

Field of invention

The present invention relates to a new multi-stage-multi-active-agent-whitening procedure for teeth, comprising the use of novel enzymatic and novel chemical whitening formulations; it also comprises the use of novel stain weakening enzymatic formulations, resulting in stain components that exhibit an increased response to whitening by oxidative whitening chemicals. The new whitening agents can also be used in single stage whitening procedures.

Background of the invention

Many people have a natural desire for sparkling white teeth. In reality, however, they are often coloured and stains may have appeared on the surface. The origins have been well documented and a distinction is made between extrinsic stains, appearing on the surface of enamel or in the dentin close to the surface; and intrinsic stains, located more deeply inside the tooth structure. Extrinsic stains can be caused by bacterial debris, build-up of glycoproteines, and precipitation of chromophoric molecules . Other sources involve degradation products from blood, hemoglobuline and sulfur containing proteins or the use of medicines that contain chlorhexidine, iron, mangan or silver containing compounds. Another important source of extrinsic stains are foods such as coffee, wine, tee or certain fruits (cherries, resins). Especially lipophilic polyphenolic food ingredients easily form complexes with proteins and starches and precipitate on the surface of teeth, to form yellow brown stains. Over time they can penetrate deeper into fissures and be very difficult to remove.

Also nicotine and tar from cigarettes and cigars contribute to the staining process.

At least part of the extrinsic stains can be removed successfully by abrasives and mechanical brushing; bleaching compositions, either with chemicals or enzymatic bleaching agents, can help though.

Intrinsic stains may have endogenic causes; they may be the result of degradation products from the body such as blood, consumption of tetracycline's, fluorides, or intake of food components that reach the teeth from the blood vessels. They can also be caused by certain diseases such as sickle-cel anemia, thalassemia and diseases of kidney or liver. These stains can only be removed by agents able to penetrate into the teeth.

Over the years a variety of bleaching products and procedures have been developed.

Almost all of them rely on the use of (hydrogen)peroxides. In some cases, peroxide & sodium perborate mixtures are used. Commercial bleaching products for use at home contain the popular carbamide peroxide. Typically it contains approx. 10 % carbamide peroxide which equals 3 % active hydrogen peroxide. Products with higher concentrations are available for use by the dental profession.

Concentrations up to 35 % hydrogen peroxide are in use.

The whitening performance and the required number of treatments depends primarily on the peroxide concentration. As home bleaching products have relative low dosages of peroxide, frequent repetition of treatments over many days and even weeks is required, before whitening appears and carbamide peroxide concentrations below 10 % show modest performance anyhow. Peroxides become more effective at higher concentration but simultaneously toxicity goes up and side-effects surface.

Kwong et al. (NZ Dent. J. 89:18-22,1993) observed a moderate inflammatory pulpal response in teeth extracted after application of carbamide peroxide over a period of two weeks.

Cherry et al. (J.Dent.Res. 72:1298-1303,1993) showed that ingestion of carbamide gel (10 % to 35 %) resulted in toxic effects in female rats. Respiration frequency and body temperature decreased substantially. They also observed eye closure, blood in urine and incontinence. Some animals died of gastric hemorrhaging. Seghi and Denri (J.Dent.Res.71:1340-1344,1992) observed that enamel treated with bleaching gel exhibited a small decrease in abrasion resistance. And Pinheiro et al. (Braz. Dent. J.,7(2), 75-79,1996) found that the micro hardness of the enamel was reduced substantially; samples with natural micro hardness of 235 (Vickers micro hardness units) dropped to 208 and 170 Units after bleaching with respectively 10 % and 16% carbamide peroxide, resulting in increased brittleness and weakness. Peroxides are not selective in their oxidation reaction. They do not only react with the staining chromophores, but also with other bio-molecules that are present inside the teeth, molecules that are not contributing to colour formation and that preferably should remain untouched. Peroxides are especially keen to oxidize saccharides. In addition, peroxides irritate gums and throat, react with essential amino acids and with dental restorative materials. Regularly, patients perceive their teeth as painful and sensitive after a bleaching session.

In order to minimize side-effects, limitation of the dosage of use is required, but then again this reduces bleaching efficiency; a compromise dosage has to be accepted between performance and safety.

Alternative bleaching products and procedures that exhibit outstanding bleaching performance, while demonstrating enhanced safety through limitation of use of peroxides or elimination altogether, would be of major interest. They would

constitute the long searched ultimate combination of "performance in safe use". That is why there is a need for new creative bleaching solutions.

Recently enzymes have entered the field as alternatives to peroxides. Potentially they offer a higher safety level because they are used at extreme low concentration and they exhibit selectivity with respect to their substrates ; however this selectivity accounts also for the inability to provide full bleaching performance, given the diversity of components in stains.
Any effective enzymatic treatment will therefore require the use of a variety of different types of enzymes, all attacking specific ingredients of the stain, until it can totally disappear. Only a multi-enzyme treatment procedure can exhibit the potential of combining high bleaching efficiency and safety.

Prior art.

Papain enzyme.

WO 99/25315 patent application claims whitening capabilities for a toothpaste comprising the papain enzyme from the papaya plant.
Japanese patent application JP08157352 claims the use of a broad range of cleaning compositions comprising a thiol containing protease in a deactivated form; deactivation is said to improve shelf life and storage stability.

Laccase/hydroxybenzotriazool (HBT)

WO97/06775 and US5989526 claim compositions comprising oxido-reductases, in particular laccases in combination with an activating co-ingredient, that are able to whiten teeth in a one-stage treatment procedure.

Summary of the invention

The main advantage of the invention is the discovery of new bleaching procedures for teeth that provide the desired combination of increased whitening efficiency with enhanced safety standards as a result of the use of very low concentrations of active agents.

We have, surprisingly, discovered that the tannase enzyme as well as the papain enzyme (in the presence of activating co-ingredients) is able to whiten teeth effectively. We have also discovered that a pre-treatment with the laccase enzyme and with hydroyxybenzotriazole (HBT) increases the vulnerability of stain components to bleaching with oxidizing chemicals. We have also found that dioxiranes have a strong bleaching effect and we have discovered that the combined use of these novel agents in a multi-stage-multi-active-agent-procedure and in an optimal order of sequence, provides synergy and delivers the combination of enhanced whitening capabilities at extremely reduced concentrations of active ingredients, a combination of desired properties not matched by products or bleaching procedures that are currently known (peroxides).

Brief description of the figures.

Fig.1 contains data from experiment 3.

It shows the influence of a laccase/HBT treatment on the bleaching performance of hydrogen peroxide. The y-axis shows the increase in blue pixels resulting from the bleaching experiment. The x-axis shows the five consecutive bleaching treatments on two teeth halves.

Fig.2. contains data from experiment 4.

It shows the influence of a laccase/HBT pre-treatment on the bleaching performance of hydrogen peroxide. The y-axis shows the increase in blue pixels resulting from a bleaching treatment. The x-axis shows four consecutive bleaching treatments on two teeth halves.

Fig.3. contains data from experiment 5.

It shows the synergistic influence of a laccase/HBT treatment on the bleaching performance of hydrogen peroxide. The y-axis shows the increase in blue pixels resulting from a bleaching treatment. The x-axis shows two consecutive bleaching treatments on two teeth halves.

Fig.4. contains data from experiment 7.

It shows the whitening performance of dimethyl-dioxirane. The y-axis shows the increase in blue pixels from a bleaching treatment. The x-axis shows the dioxirane treatments (1 and 2) and the hydrogen peroxide treatment (3).

Terminology

Whitening & bleaching.

In the context of this document, the meaning of terms "whitening" and "bleaching" is the same and referring to a change in the degree of whiteness of teeth.

Whitening composition.

The words "product", "composition" and "formulation" have the same meaning. A whitening composition is a formulation that has the capability, by action of physical contact, to enhance the degree of whiteness of teeth.

Stain weakening composition.

This formulation does not have the capability to whiten , when it is in physical contact with teeth. However, a stain weakening composition has the ability to change the molecular structure of stain components (without actually changing the degree of whiteness of the tooth), resulting in an enhanced response of the stains during the next and separate treatment stage with a whitening oxidation chemical, such as hydrogen peroxide. In other words a pre-treatment with a "stain weakening composition" acts synergistically upon the next treatment stage and will enhance the performance in the next and separate treatment stage of the whitening formulation with the oxidation chemical.

Single stage bleaching procedure.

Single stage refers to a procedure where the teeth are subjected to only one bleaching composition and the total whitening procedure consists of only one treatment step (or stage).

Multi stage bleaching procedure.

Teeth are exposed to two or more bleaching steps (or stages) that are carried out separately, one step after the other. In every treatment step the teeth are exposed to a composition.

The different treatment steps may comprise the use of an identical composition. Hence, exposure of teeth to this composition is repeated. This is called "multi-stage-mono-active-ingredient-bleaching procedure".

Alternatively, the steps may comprise the use of different non-identical formulations, with different active ingredients, which is called a "multi-stage-multi-active-agent-bleaching procedure.

The formulations may refer to "whitening"- as well as "stain weakening compositions".

Delivery system

Whitening and stain weakening compositions can be administered to teeth in a variety of delivery forms: tooth paste, gel formulation for use in a plastic tray that covers teeth, mouth washes, dental creams , dental cleaning agents, chewing gum, patches. Each delivery system is specific in terms of ingredients, concept of administration to teeth, and contact time. For example a toothpaste typically contains 10 to 70 % abrasive ingredients, is applied by a tooth brush and the contact time is seconds up to a few minutes. A whitening gel does not contain an abrasive, it is applied by a plastic tray that covers the set of teeth and the treatment time can be as long as the whole night.

With respect to products that are used at home, it is traditional practice to use a formulation with carbamide peroxide, repetitively used (in several treatment stages) and over a longer time period as a gel formulation for use in a plastic tray. This is called a "multi-stage-mono-active-ingredient-bleaching-procedure" and "a gel formulation & plastic tray" delivery system.

Administration.

The term "administration of a composition" means the arrangement of physical contact between a composition and teeth, in the form of a selected delivery system and for a selected treatment time period.

Description of the invention

This invention is about novel innovative whitening products, based on enzymatic and non-enzymatic active ingredients; products that, when used together within the context of a "multi-stage-multi-active-agent-bleaching-procedure", exhibits a better bleaching performance and superior safety conditions, as when compared to the use of traditional whitening products such as hydrogen peroxide or carbamide peroxide.

A method for the measurement of the degree of whiteness has been developed. Traditionally, the whiteness of teeth is assessed either by visual inspection (comparison with standard teeth having different degrees of colouration; Vitapan shade guide index) or by means of diffuse reflectance spectrophotometry (L*a*b*).

The first method has limitations. Regularly, the base-colour (i.e. the colour that is uniformly spread over the total surface), is supplemented with local yellow/brown spots and stains of a different kind and origin.

Bleaching agents do not necessarily exhibit similar bleaching capabilities on different types and parts of the stain and a differentiation cannot be made accurately since the shade standards (made from glass-ceramic material) are coloured uniformely. Identification of a colour change in a local stain spot is difficult to carry out.

The second method suffers from the same limitation . An average degree of whitening can though be measured for a particular part of the tooth. It is however not straightforward to position the tooth on the measuring device in such a way that always the same part of the tooth is under analysis.

Prof. C. Bently et al. (J.Amer.Dent.Assoc. vol 130/06,1999,809) has demonstrated the possibility to monitor changes in tooth brightness with photographic equipment. They discovered a good relationship with the mean pixel intensity for the RGB blue channel (MPI-b) on a picture. This is of no surprise because blue is the opposite colour of yellow, the dominating colour of stains on teeth. This parameter appears to provide a better brightness descriptor than when using reflectance spectrophotometry and better correlation with the Vitapan shade guide. In order to avoid large standard deviations as a result of variations in the properties of the films, we have made use of a sensitive digital camera, Nikon coolpix 990.

This method enables the detailed study of every little stain spot present on a tooth surface with a high degree of reproducibility. In the RGB system the intensity of the colour is defined on a scale from 0 (black) to 255 (white). Under our camera setting, the standard teeth from the Vitapan shade guide vary from approximately 100 MPI-b (tooth 5M3) to 200 (tooth 1M1).

Tannase.

In one aspect of the invention we have surprisingly found that the enzyme tannase (tannin acylhydrolase ED 3.1.1.20) is capable of increasing the whiteness of teeth.

Extrinsic stains are a mix of bacterial debris, proteins and polymeric polyphenolic structures, which are very difficult to remove. The strategy is to break down polymeric material and complexes , either by hydrolyses or oxidation. The smaller the compounds become, the more readily they will disappear from the teeth.

Tannase hydrolyses gallic acid from tannin and contributes to the degradation process. Exp. 1 demonstrates it's ability to increase whiteness from

13 to 38 blue pixels (change of MPI-b) especially on local stain spots on the tooth surface.

Tannase can be used within a pH range of 2 to 9, preferably 4 to 7 and a reaction temperature between 0 C and 80 C, preferably 35 C to 45 C. The enzyme concentration and activity can be anything (without limitation) and may vary widely between below 1 U/ml to more than a couple of thousand units/ml bleaching formulation. The Unit of Activity is according to the suppliers method (Kikkoman Corp.).

Tannase may be derived from a traditional source such as Asp.oryzae or from other sources or may be genetically engineered.

Tannase should preferably (but not necessarily) be used in combination with activating co-ingredients such as EDTA, cysteine and a thiol containing molecule (e.g. mercaptoethanol). Concentrations may vary widely, but 0.25 % EDTA 0.5 % cysteine and 0.03% mercaptoethanol w/w in the bleach formulation are indicative.

The treatment with tannase can preferably be preceded by a treatment with the papain enzyme.

Initial removal of proteins and bacterial debris clears layers of tanninic structures in such a way that they become more vulnerable towards attack from the tannase enzyme.

The enzyme can be used within a formulation that does not contain another bleaching agent; as such it can be used as an enzyme bleaching step in a "multi-stage-multi-active-agent-bleaching" procedure. It can also be used in a formulation that contains other bleaching agents such as oxido reductases (in particular laccases), proteases (papain), lyzozyme, or in combination with traditional chemical oxidizing agents such as hydrogen peroxide, carbamide peroxide, sodium perborate,...

Papain

In another aspect of the invention we have surprisingly found that the papain enzyme demonstrates substantially enhanced bleaching activity, when activating co-ingredients are added to the bleaching composition.

Although patent application WO99/25315 claims whitening capabilities for a toothpaste comprising the papain enzyme from the papaya plant, we have discovered, surprisingly, a new formulation comprising the papain enzyme, but having far greater whitening capabilities compared to WO99/25315, due to the presence in our formulation of co-ingredients that have the ability to boost the enzyme's whitening activity.

We have, surprisingly, found that , despite the strong attachment of stain components on the tooth surface and the strong complexation phenomena between stain components (proteins and polyphenolic stain components generate complexes and precipitate on tooth surfaces) papain does become a more effective whitener when applied in combination with activating co-ingredients such as cysteine, EDTA and a thiol containing compound.

These co-ingredients are not present in the formulations mentioned in WO99/25315. Although our invention can be used in many delivery systems such as toothpaste, mouth washes and others, we also prefer, describe and contemplate the use of a whitening formulation (comprising the papain enzyme and it's activating co-ingredients) in the form of a gel for use in a plastic tray that is applied as a cover on teeth. This procedure allows longer treatment times (hours) compared to the use of toothpaste (minutes) and this further increases the whitening capabilities. Gels and toothpastes are different technologies, having completely different ingredients and serve a different purpose. The gel/tray delivery system has not been described in patent application WO99/25315.

Japanese patent application JP 08157352 describes formulations for use in a broad range of cleaning applications (bath preparations, toothpaste, general cleaners) and comprising thiol-containing proteases that have been added in a deactivated form in order to achieve improved shelf life during storage. The enzyme is said to regain activity during the use of the formulation as a result of the occurrence of small amounts of products such as cystine in the water, originating and released from the living body. However, the patent does not mention formulations comprising the addition of products such as cysteine, EDTA, thiol containing products; the patent does not mention the application of tooth whitening or the effect of the co-ingredients on tooth whitening performance, nor does it refer to the use of a gel/tray delivery systems, a preferred system for whitening products. The patent does not describe compositions comprising activated proteases whereas our formulations comprise activated papain.

Overall, our whitening formulation and preferred delivery system offers superior whitening performance compared to existing formulations and none of the formulations known in the public domain for use as whitening compositions are using papain in combination with activating ingredients.

Experiment 2 demonstrates a whiteness increase of 14 blue pixels when using a bleaching formulation with papain as well as additional activating co-ingredients, and no pixel increase when activating co-ingredients where not present.

In another part of the experiment, respectively the values 9 and 3 blue pixels have been obtained.

The papain latex from the Carica papaya plant contains broadly active proteases, lyzozyme and esterase's.

It does whiten both the basic colour of the teeth as well as local stains. It's action is supported by the use of tannase. Suitable concentrations for activating ingredients are 0.25 % EDTA, 0.5 % cysteine hydrochloride and 0.03% mercaptoethanol.

Other concentrations can be used effectively. Mercaptoethanol can be replaced by other thiol containing compounds (e.g. dithiotreitol); metabisulfite salts and N-bromo-succinimide can also contribute to the activation process. The papain enzyme can be used between a pH range of 4 and 9, preferably 6 to 8; it is active between 10C and 80 C. The concentration of papain may vary widely. An appropriate (but not limiting to) concentration is 50 mg papain / 10 ml bleaching solution from an enzyme with 30.000 USP/mg activity. Other concentrations and activities can be used too. The performance of bleaching formulations with papain & co-ingredients varies with the tooth sample; MPI-b changes between 12 and 40 pixels have been found. In most cases a repetition of the papain treatment provides an additional whiteness gain.

Papain may be replaced by proteases from other sources, but they are less reactive. Papain (and it's activation co-ingredients) may be used in a formulation and this eventually in conjunction with other enzymes such as tannase, laccase, other oxido-reductases, lyzozyme,....or mixed with classical oxidizing agents.

Although papain can be used in toothpastes, it is preferable to use delivery systems that allow a longer treatment time such as bleaching gels (for use with trays) or ready made patches loaded with an enzymatic bleach composition.

Laccase

Patent WO97/06775 and US5989526 claim the use of a formulation comprising an oxido-reductase enzyme for whitening of teeth in a one stage treatment procedure. Especially laccases are contemplated and an additional co-product,

so-called "mediator" is added to improve the bleaching performance. Supposedly the whitening is resulting from the direct contact of the enzyme and/or mediator with the stains on the teeth.

However we have discovered and experimentally demonstrated that laccases alone or laccases in combination with the mediator, hydroxybenzotriazole (HBT), do not whiten teeth at all. We have also discovered surprisingly that the laccase/HBT combination can be used for another interesting property.

A treatment with laccase/HBT changes the chemical structure of stain components (without whitening). We have also found that such treatment acts synergistically with a subsequent and separate oxidation treatment step. In other words, the laccase/HBT pre-treatment has weakened the structure of the stain components in such a way that the stain itself has become more vulnerable towards a subsequent whitening treatment step with an oxidation chemical such as hydrogen peroxide, carbamide peroxide or dioxirane.

There is no mention in the patents WO97/06775 and US5989526 of the existence of such a synergistic effect nor does the patent comprise a multi-stage procedure with an oxidation treatment step preceded by a non-whitening laccase treatment step, in order to take advantage of the synergy. The laccase/HBT combination does not act as a whitener but as a "stain component weakening agent".

The use of a pre-treatment step with laccase/HBT in a two-step whitening procedure, does increase the whitening performance of the subsequent oxidation treatment step with 10 to 80 % (on average 40 %) on natural teeth; on teeth that have been stained deliberately, increases up to 300 % have been noticed.

The following experiments are demonstrative:

Experiment 3: tooth A and B are different teeth. Peroxide treatments provide tooth A with the highest increase in whiteness only in bleach procedures where A has also been subjected to a laccase pre-treatment. In cases where A and B,

both, have been treated or not have been treated with laccase, A isn't whitening more than B.

Experiment 4 and 5: A similar experiment has been carried out with two teeth halves from the same tooth. There is no difference in whiteness gain in the absence of a laccase treatment. In all cases where A has been subjected to laccase, it's gain in whiteness has become 50 % more effective compared to tooth halve B.

Experiment 6: The teeth from experiments 3,4,5 have been stained on purpose with chlorhexidine and tea. In this experiment naturally stained tooth halves have been used. The same strong synergistic effect between laccase and hydrogen peroxide could be noticed.

Conclusively, laccase/HBT can be used effectively as a "stain structure weakener" in a multi-stage-bleaching-procedure.

On the basis of this, newly discovered, synergistic effect, we claim a multistage bleaching process (comprising at least two steps or more) in which a peroxide bleaching step is preceded with a laccase/HBT treatment in order to take advantage of the synergy.

Laccase can be used in a concentration between 0.0001% and 25 % based on end weight of the bleaching formulation. The pH can be between 4.5 and 9, preferably between 4.6 and 7.5. Laccase can be supplemented with other enzymes such as tannase, glucoseoxidase or oxidoreductases such as Mn peroxidase. The laccase solution can further be saturated with oxygen.

The laccase can be obtained from every known source such as for example Collybia, Fomes, Lentinus, Pleurotus, Aspergilus, Neurospora, Podospora, Phlebia, Coriolus, Botrytis, Polysporus sp., M. thermophilia, Rhizoctonia sp., Rhus sp.

Dioxiranes

In another aspect of the invention we have discovered that dioxiranes are able to whiten teeth. They are composed of a three-atom-membered ring structure with two oxygen and one carbon atom. They are strong oxidizers (dimethyldioxirane is ten-fold more reactive compared to hydrogen peroxide).

They are of particular interest because they combine bleaching capabilities at a low dosage with lipophilic properties. Hydrogen peroxide does not preferably react with lipophilic stain components because it exhibits hydrophilic properties. Dioxiranes will react more readily with lipophilic stain components, which are the most difficult to remove.

Experiment 7 shows the effectiveness of dimethyldioxirane at dosages substantially lower than in use for hydrogen peroxide. First dioxirane treatment delivered a rise with 29 blue pixels and in another occasion (experiment 8) 28 blue pixels.

Dioxiranes can be made in situ from a ketone catalyst and oxone (ref. Curci et al., J. Org. Chem., 1980, 45, 4758; Kurihara et al., tetrahedron Lett., 1994, 35, 1577 ;Denmark et al., J.Org. Chem., 1995, 60 ,1391 ; Yang et al., J.Org. Chem. 195,60,3887). Production preferably is carried out between 0C and 40 C; The pH is between 6 and 8, preferably around 7 and a buffer such sodium carbonate (or caustic soda) is used to neutralize the acid of oxone. The reaction is carried out in water and an appropriate ketone should be used such as acetone, cyclohexanone, 1,4-cyclohexane-dione, cyclic ketones with more than one cyclic ring structure or bis(triethylphosphine)-platinum (II)-oxalate.

The following concentrations are used in a preferred enbodiment: 2mmole sodium carbonate /10 ml , 1.5 mmole oxone /10 ml and 2 mmole keton /10 ml bleaching formulation.

Other concentrations can be used.

The use of dioxiranes provides a means to reduce the total chemical exposure (dioxiranes act at lower concentrations than hydrogen peroxide), and provide an increase in selectivity towards attack of lipophilic stain components.

Multi-stage-multi-active-agent-bleaching-procedure

In the unifying aspect of the invention a "multi-stage-multi-active-agent-bleaching-procedure" is claimed with better whitening capabilities compared to peroxides thanks to,

1. a synergistic action between enzymes and chemical oxidizing agents,
2. a profound whitening capability with bleaching procedures that include enzymatic stages,
3. substantial reduction in the chemical exposure when enzymes are used.

All the foregoing enzyme bleaching agents act selectively, providing better safety conditions, but it explains also why they are not able to bleach teeth completely on their own. When they are put in use all together, however, they are often able to generate completely white teeth. The enzymes alone can achieve a whiteness increase in the order of between 50 and 60 blue blue pixels. It is well known by the professionals that hydrogen peroxide (and carbamideperoxide) act predominantly on the base colour of teeth. It is very difficult (sometimes not possible) to completely remove the brown/yellow spots (presumably because they carry lot's of lipophilic material, which is difficult to remove by a hydrophilic agent).

Tannase, dioxirane and laccase do act on lipophilic moieties and contribute to complete removal of these stain spots. A procedure that includes the use of enzymes is able to provide more complete whiteness compared to the use of hydrogen peroxide or carbamide peroxide.

Papain and activating co-ingredients act predominantly on hydrophylic segments, (proteins, bacterial debris); tannase chops gallic acid from taninic components such as gallocatechins and proanthocyanidins. Preferably papain and tannase enzymes can be repeatedly used in an alternating procedure; they will exert a positive influence on each others action as they "clean" the site for the next enzyme.

Laccase/HBT continues the attack on phenolic segments of the remaining part of tanninic structures. At this stage the stains are partly removed and the remainder is weakened in such a way that lower amounts of oxidation agents (if needed at all) are sufficient to reach full whiteness. At this stage either a classical peroxide or dioxirane can be used.

The use of enzymes acts synergistically on subsequent oxidizing stages (either with dioxiranes, hydrogen peroxide or carbamide peroxide).

Tooth C from experiment 9 has been treated with the following sequence : dioxirane-hydrogen peroxide-papain-tannase-papain-laccase-dioxirane . Witeness increased with respectively 0 and 28 blue pixels after (and as a result of) the first and the last dioxirane stage. This demonstrates that the enzymes partly remove and weaken stain spots in such a way that it becomes possible for dioxirane to provide better bleaching result. The synergy can be realized with tannase, laccase or papain or a combination of them.

Another important aspect of the invention is the possibility to drastically reduce the chemical exposure, improving safety standards further. Even a treatment with a rather low amount of hydrogen peroxide (e.g. 5 % w/w) entails an exposure to teeth with approximately 1.5 mole/l. Given the very low amount of pure enzyme protein used in the treatment and it's high molecular weight, the chemical exposure from enzymes will be a factor 10.000 and more lower compared to

peroxide, on the basis of available active molecules. This is true also for dioxirane. A 9% w/w composition with oxone contains only 10 % from the mole concentration of a 5 % hydrogen peroxide solution. It is to be expected that the dioxirane concentrations will still be substantially lower than the initial oxone concentration.

On average, and when considering a complete multi-stage bleaching procedure, the new bleaching procedure provides a chemical exposure on teeth of only 1 tot 5 % compared to the classical level of exposure with peroxides, a dramatic reduction indeed.

All in all, any multi stage bleaching procedure taking advantage of enzymes and/or dioxiranes will be safer, compared to the sole use of peroxides, not only because of the lower levels of chemical exposure, but also because enzymes act predominantly on the surface of teeth and not inside.

Appropriate delivery systems

The bleaching and stain weakening formulations can be delivered in a variety of "delivery systems". Examples are gel formulations for use in a plastic tray , toothpaste, dental creams, mouth washes, denture cleaning agents, chewing gum, patch based systems.

Especially contemplated and of interest to us are "whitening and stain weakening" gel formulations for loading into a dental tray designed for placement over teeth such that the bleaching composition will contact the tooth surface. This system allows prolonged contact times between the gel formulation and the teeth, further improving the effectiveness of the whitening gel in a substantial way. Normal practice entails contact times between 10 minutes up to a few hours and up to complete overnight treatment. Toothpaste whitening

formulations exhibit much shorter contact times (seconds to minutes only) and this does not favor an optimal enzymatic whitening performance.

Typically gel formulations (for use in a plastic tray) contain the active ingredients, a thickener (such as carbopol, xanthan gum, starch, alginates, pectin, cellulose derivates, polyacrylic acid and salts, a pH adjusting agent (such as calcium carbonate, caustic soda,..), a chelating agent (such as EDTA), a humectant (such as glycerol, polyol, sorbitol, polyethylene glycol, propylene glycol, 1,3 propanediol, 1,4 butanediol, hydrogenated and hydrolysed polysaccharides), a sweetener (such as saccharine), flavors, perfume, anti-bacterial agents (such as chlorhexidine digluconate).

The gel formulation may e.g. comprise between 0.1 % and 20 % thickener (w/w); ingredients such as a chelating agent, a sweetener, flavor, perfume, an anti-bacterial agent may e.g. be present in amounts between 0 % and 5% .

The concentration of humectant can be between 0 and 80 %.

The delivery systems may be of the "one compartment type" but also included in the invention are the two-compartment formulations; for example the "stain weakening" delivery system may e.g. consist of two formulations: a laccase formulation and a formulation with the mediator for mixing just before use.

Equally the dioxirane delivery system may e.g. consist of a formulation with oxone and another formulation with the keton catalysts and the pH modifying ingredient, for mixing just before use.

The bleaching and stain weakening formulations may be included in products for use by professional dentists in office, or may be part of "dentist prescribed but home used" products.

Also contemplated are over-the-counter whitening products and kits.

In another aspect of the invention the bleaching and stain weakening formulations may be added to abrasive creams for use by the dentist.

Abrasive polishing material may include alumina based products, magnesium trisilicate, magnesium carbonate, sodium and calcium bicarbonate, kaolin, aluminosilicates, zirconium silicates, calcium pyrophosphate, other phosphates , hydroxyapatite, powdered plastics such as polyvinylchloride, polyamides, polymethyl methacrylate, polystyrene, phenol-formaldehyde, melamine formaldehyde, urea formaldehyde resins, epoxy resins, silica xerogels, and the like.

Anti-bacterial agents can be added such as chlorhexidine digluconate, hexetidine, alexidine, quaternary ammonium compounds, metal ions such as zinc, copper, silver, and stannous chloride.

Examples

A. Methods and materials

Teeth:

Teeth react differently to bleaching agents. Even teeth with similar degrees of staining exhibit a wide variation in response. This is why teeth samples have been cut into two halves and the cut surface has been closed with an acrylate layer, enabling us to do comparative studies on tooth halves with similar properties. Cutting along the long axis of the tooth, in mesio-distal or bucco lingual direction has been performed. All tooth parts are large enough for close analyses with the digital photographic method.

Staining.

Some tooth samples have been stained on purpose by submerging in a 10 ml mixture of chlorohexidine di gluconate (0.5 % w/w), tea and coffee components, and human saliva. Samples have been submerged five times at room temperature for a period of one day and have been dried in between by air.

Determination of tooth colour by mean blue pixel value.

The degree of whiteness can be determined by measuring the mean blue pixel intensity (MPI-b) on a picture according to the method of Prof. C. Bently (1999). It appears that MPI-b exhibits a better correlation with the Vitapan shade guide than data from reflectance spectrophotometry.

In order to avoid variations caused by using photographic films with varying properties, we have taken pictures with a digital Nikon 990 coolpix camera (3.300.000 pixels).

Digital image processing and analyses has been carried out with commercially available software.

Reflexions (rarely present) where digitally removed from the photographs and the Mean Blue Pixel Intensity (MPI-b) has been measured either on the complete tooth surface or on local stains. This value reflects the whiteness of the tooth. In the RGB colour system , colours are defined on a scale of 0 (black) to 255 (white). The difference MPI-b between the standard tooth 1M1 and 5M3 is approximately 100 pixels.

If camera settings are kept constant, the standard deviation is limited (2 pixels). The state of dryness has a substantial effect on the whiteness appearance. Tooth samples have been stored in deionised water. Just before taking the picture, the water on the surface has been removed by a paper towel; the picture was immediately taken within ten seconds.

In order to account for possible variations due to camera settings, five standard teeth from the Vitapan shade guide (1M1, 2L2.5, 3M.3, 4M3 and 5M3) have also been added to the picture.

Enzymes

Laccase is from trametes hirsuta and has an activity of 14000 nkat/ml.

1 Unit (16.7 nkat) is the amount of enzyme that catalyses the conversion of 1.0 µmole ABTS per minute at pH 4.5.

Tannase enzyme is from Asp. Oryzae and has an activity of 5000 U/mg (supplier Kikkoman Corp.)

Papain enzyme is from Carica Papaya L and has an activity of 30.000 USP U/mg. 1 Unit is the activity that releases the equivalent of one µg of tyrosin from a specified casein substrate at 40 C, pH 6 and within 60 minutes period.

B. Results

Experiment 1 (tannase)

Tooth samples have been subjected to a treatment with the tannase enzyme (tannin acylhydrolase EC 3.1.1.20) having an activity of 5000 U/gr (determination of activity according to the supplier Kikkoman). The 10 ml aliquot contained a citrate buffer at pH 5 or 4.7 as well as 0.2 % EDTA, 0.45 % cysteine hydrochloride and 0.0025 % mercaptoethanol. Temperature was 37C and reaction time 2 hours.

Sample no.	Tannase	pH	Blue pixels	Gain in blue pixels	
	mg/10 ml		before reaction total surface (1)	after reaction total surface (2)	after reaction local stain (3)
1	100	5	186	9	38 / 21
2	100	4.7	183	7	22
3	50	5	205	3	14 / 16
4	50	5	157	9	13

(1): level of whiteness in blue pixels (MPI-b) before the reaction with tannase.

(2): the amount of additional blue pixels (change of MPI-b) on the total tooth surface due to tannase.

(3): the amount of additional blue pixels on local stained spots present on the tooth surface.

All of the tooth samples have been pre-treated with a bleach formulation containing the papain enzyme and activating co-ingredients. At this stage, though, they have not yet been completely free from local stain spots.

The tannase acts predominantly on these yellow/brown spots. The colour reduction can be seen by visual inspection as well as measured in average mean blue pixel intensity MPI-b.

The amount of acid present (pH 5) is too low to account for a contribution to the whitening process.

This is demonstrated also in experiments 9A and 9D. Furthermore, the presence of proteins appear to have a preventive effect on acid hydrolyses.

Experiment 2 (papain treatment; role of activation ingredients)

Two tooth halves with natural staining have been used.

Pixel values for untreated halves are 119 (A) and 132 (B) (standard teeth from the "Vitaplan 3D-master tooth colour guide" have 136 (4M3) and 102 (5M3) pixels.

Both halves have been treated twice with refined papain enzyme (30.000 USP/mg) in 10 ml phosphate buffer at pH 7. The reaction mixture of tooth A contained EDTA (0.4 % or 0.2 %), cysteine.hydrochloride (0.9 % and 0.45 %) and mercaptoethanol (0.005 % and 0.0025 %), respectively in the first and second treatment stage.

The reactions where carried out at 37 C, for 2 hours.

	Tooth halve A	Tooth halve B
Pixel value of native tooth halve	119	132
Pixel gain after first treatment	14	0
Pixel gain after second treatment	9	3

The whiteness increase of tooth halve A is superior to B, due to the presence of activating co-products in the bleach formulation.

Experiment 3 (laccase, two different teeth, natural stains)

Two different teeth with natural stains have been subjected to five consecutive hydrogen peroxide treatment stages.

The peroxide treatments no. 2,3,4 and 5 of tooth A (left side bars), have been preceded by laccase treatment stages.

As for B (bars in fig. on right hand side), only peroxide treatment no.5 has been preceded by a laccase treatment.

Peroxide treatment: 22 mg EDTA, 4.6 % w/w H2O2, pH 7.4, temperature 37 C, 2 hours.

Laccase treatment: 15 ml buffer pH 4.8 (citrate;Sigma) 10 minutes saturation with oxygen gas, hydroxybenzotriazole 4mM, 8.5 to 12 U hirsuta trametes laccase, 38 C, 2 hours.

Fig.1 and the table show the pixel gains from the peroxide treatment steps only:

Pixel gain of ..	tooth A	tooth B
Peroxide bleaching step 1	6	9
step 2	19	15
step 3	13	9
step 4	7	3
step 5	11	14

See also Fig. 1

The whiteness increase from a peroxide treatment on tooth A is superior to tooth B after treatments no. 2, 3 and 4, as only tooth A had been subjected to a pre treatment with laccase.

The extra whiteness gain for tooth A over B is 26%, 44 % and + 100 % (per step).

When there is no pretreatment with laccase (1) or when both teeth are treated with laccase (5), the results from tooth A are not anymore better compared to B.

Experiment 4 (laccase, one tooth, stained deliberetaly)

A tooth has been cut in two parts and the cut surface has been closed with a polyacrylate layer.

Both halves have been stained with tea and chlorohexidine.

The whiteness values of the stained halves where 115 and 108 blue pixels (to be compared with 198 for 1M1, 123 for 4M3 and 87 for 5M3 standard teeth from VITAPAN shade guide).

The left tooth halve (ref. Fig. 2) has been subjected to pre-treatments with laccase before bleaching with hydrogenperoxide (in steps 2,3 and 4).

The right tooth halve has not been subjected to a laccase treatment.

Peroxide treatment: 22 mg EDTA, 4.6 % w/w H2O2, pH 7.4, 35-40 C, 2 hours.

Laccase treatment: 15 ml buffer pH 4.8 (citrate;Sigma) 10 minutes saturation with oxygen gas,

hydroxybenzotriazole 4mM, 8.5 to 12 U hirsuta trametes laccase, 38C, 2 hours.

Pixel gain of..	left tooth halve	right tooth halve
Peroxide bleaching step 1	13.75	13.0
step 2	11.7	4.9
step 3	6.2	1.1
step 4	4.9	1.6

See also Fig. 2

With respect to the first treatment with hydrogen peroxide there is little difference between the tooth halves, as none have been pre-treated with laccase. The bleach results from the peroxide treatments 2,3 and 4 of the left tooth are superior, due to the laccase pre-treatment.

Experiment 5 (laccase, tooth halves of same tooth, stained deliberetaly)
The tooth has been cut in two halves and the surface has been closed with a polyacrylate layer.
Both halves have been stained with tea, coffee and chlorohexidine.

The whiteness values of the halves where 141 and 122 blue pixels (to be compared with 1M1:187, 2L2.5: 168, 3M3: 150, 4M3: 125, 5M3: 93 standard teeth from the VITAPAN shade guide).
The left tooth halve (ref. Fig. 3) has been subjected to a pre-treatment with laccase before bleaching with hydrogen peroxide in step 2 only.
The right tooth halve has not been subjected to a laccase treatment.
Peroxide treatment: 22 mg EDTA, 3.5 % (stage 1) and 4.6 % (stage 2) w/w H2O2, pH 7.5, 35-40 C, 2 hours.

Laccase treatment: 15 ml buffer pH 4.8 (citrate;Sigma) 10 minutes saturation with oxygen gas,
hydroxybenzotriazole 4mM, 8.5 to 12 U hirsuta trametes laccase, 38C, 2 hours.

Pixel gain of..	left tooth halve	right tooth halve
Peroxide bleaching step 1	10.4	11.1
step 2	16.5	4.3

See also Fig. 3

The bleaching result of the left tooth is superior in stage 2, thanks to the laccase pre-treatment.
In stage 1, none of the halves have been treated with laccase.

Experiment 6 (laccase, toothhalves of same tooth, natural stains)

The tooth has been cut in two halves and the surface has been closed with a polyacrylate layer.
The halves have kept their natural staining condition.

The whiteness values of the halves are 94 and 96 blue pixels (to be compared with 4M3: 123, 5M3: 90 standard teeth from the VITAPAN shade guide).
The left tooth halve has been subjected to a pre-treatment with laccase before bleaching with hydrogen peroxide in both steps (1,2).
The right tooth halve has not been subjected to a laccase treatment.
Peroxide treatment: 22 mg EDTA, 4.9 % (stage 1) and 6 % (stage 2) w/w H2O2, pH 7.5, 38-40 C, 2 hours.

Laccase treatment: 15 ml buffer pH 4.8 (citrate;Sigma) 10 minutes saturation with oxygen gas,

hydroxybenzotriazole 4mM, 8.5 to 12 U hirsuta trametes laccase, 38C, 2 hours.

Pixel gain of..	left tooth halve	right tooth halve
Peroxide bleaching step 1	32	13
step 2	30	16

The whiteness increase of tooth halve A is superior to B thanks to the pre-treatment with laccase.

Experiment 7(dioxirane)

A tooth with natural stains (whiteness value: 142; standard teeth 3M3: 163 and 4M3: 138) has been subjected to two dioxirane treatments and one hydrogen peroxide bleaching step.

The dioxirane treatment has been carried out in 10 ml deionised water with Na2C03.10H2 (550 mg or 985 mg), oxone (920 mg or 1380 mg) and acetone (100 mg and 150 mg) (amounts respectively used in the first and second dioxirane treatment).

Sodium carbonate and oxone have been mixed at 5 C; after 2 minutes the acetone has been added; after 30 minutes the formulation was allowed to heat up to room temperature and after another hour the temperature has been raised to 37 C- 40 C for another hour. (Sequence of addition of ingredients can vary).

The hydrogen peroxide reaction has been carried out in 10 ml with 22 mg EDTA, 5.3 % H202 w/w at a pH 7.5 for 2 hours.

The dimethyl dioxirane is improving the whiteness with more than one standard tooth shade in the first treatment (29 blue pixels). See also Fig. 4

Experiment 8(dioxirane)

A tooth sample with a high whiteness value has been subjected to one dioxirane treatment (10 ml, 570 mg Na2C03.10H20, 920 mg oxone, 100 mg aceton, gradual temperature rise from 5 C to 37 C over 2.5 hours.

(1M1: 192 pixels, 2L2.5:176 , 3M3 :163)

Whiteness value before dioxirane treatment	170 pixels
Whiteness value after dioxirane treatment	198 pixels
Difference	28

Experiment 9(multistage bleaching procedure)

The novel agents have been used in a multi stage bleaching procedure. The aim is to bleach teeth to full whiteness and at the same time reducing the chemical exposure to a level below the one traditionally used with hydrogen peroxide and carbamide peroxide. This enables to combine bleaching efficiency with enhanced safety procedures.

Acid treatment has been carried out with a citrate buffer at pH 5 at 37 C for 2 hours.

The papain bleaching formulation (10 ml deionised water) contained 50 mg refined papain, 0.2 % EDTA, 0.45 % cysteine and 0.0025 % mercaptoethanol; reaction at 38C for 2 hours.

The tannase treatment was carried out in 10 ml citrate buffer pH 5 in the presence of 0.2 % EDTA, 0.45% cysteine, 0.0025% mercaptoethanol and 50 to 100 mg tannase (5000U/g); reaction at 38 C for 2 hours.

The laccase treatment has been done in 10 ml citrate buffer pH 4.7, 4 mM hydroxybenzotriazole, 100 µL solution of laccase hirsuta trametes (= 560 U/l). The solution has been saturated with oxygen for a period of 10 minutes; reaction at 38 C for 2 hours.

Dimethyldioxirane has been prepared in 10 ml deionised water; at 5 degrees C 550 mg Na2C03.10 H20 and 920 mg oxone and 100 mg acetone have been mixed. After 30 minutes the mixture was allowed to reach room temperature. After another hour it was heated up to 37 C for another hour.

Hydrogen peroxide has been prepared in 10 ml aliquots of water with 22 mg EDTA, 4.8 % H202 at a pH of 7.5; reaction at 37 C for 2 hours.

Tooth halves A1 and A2 (same tooth)

Whiteness value A1: 156 Whiteness value standards 1M1: 190
at start A2: 157 Vitapan shade 3M3: 162
4M3: 140

Whiteness value A1: 196
after reactions A2: 193

Reaction sequence	*Whiteness gains in blue pixels*	
	A1	A2
1. Acid treatment	-9	-8
2. Papain & co-ingredients treatment	21	25
3. Papain & co-ingredients treatment	19	12
4. Tannase	9	7

Tooth B

Whiteness value B: 159 at start

Whiteness value standards 1M1: 192
3M3: 164
4M3: 141

Whiteness value B: 208
at end of reaction

Reaction sequence	*Whiteness gains (blue pixel)*	
	total tooth surface	Local stain spot
1. Papain & co-ingredients	40	
2. Papain & co-ingredients	6	
3. Tannase	3	7 and 8

Tooth C

Whiteness value C: 101 blue pixels at start

Whiteness value standards 1M1:192
4M3:141
5M3:111

Whiteness value C: 196
at end of reaction

Reaction sequence	*Whiteness gain in blue pixel*
0. Dimethyldioxirane	- 1.8
1. Hydrogen peroxide 4.8 %	12
2. Papain & co-ingredients	33
3. Tannase	18
4. Papain & co-ingredients	6
5. Laccase & dioxirane	28

Tooth D

Whiteness value D: 130 Whiteness value standards 1M1 : 196

Vitapan shade guide 2L2.5:173

Whiteness value D: 200

at end of reaction

Reaction sequence	*Whiteness gain in blue pixels*
1. Papain & co-ingredients	27
2. Tannase	9
3. Acid treatment	- 2
4. Papain & co-ingredients	10
5. Laccase + dioxirane	26

The examples demonstrate that a substantial part (and often the total part) of the desired whiteness gain can be achieved with enzymes, in particular with "papain & activating co-ingredients", tannase and the laccase enzyme.

The total chemical exposure, and in particular the one from oxidizing agents has been decisively reduced, adding to the safety standard of the procedure.

Data on the dioxirane treatment on tooth sample C demonstrate that preparatory treatment with enzymes remove so much components and weaken stains in such a way that they exert a synergistic effect on a subsequent oxidation treatment (pixel gain dioxirane treatment before enzyme treatments = -1.8; After enzyme treatments = 28).

Patent claims

1. A multi-stage bleaching procedure for teeth, comprising the administration of separate formulations in separate stages to teeth, whereby at least one, or more, of the stages comprise the administration of:

- a formulation comprising the enzyme papain and activating co-ingredients and/or
- a formulation comprising the enzyme tannase and/or
- a formulation comprising the stain weakening components laccase and hydroxybenzotriazole and/or
- a formulation comprising dioxirane.

2. A multi-stage bleaching procedure with at least two separate bleaching stages, whereby the first stage is acting synergistically on the next stage, and whereby a stain weakening formulation that comprises the enzyme laccase and hydroxybenzotriazole, is administered to teeth in the first stage and whereby a formulation that comprises an oxidation chemical is subsequently and separately administered in the next bleaching stage.

3. A multi-stage bleaching procedure with at least two separate bleaching stages, according to claim 2, whereby the oxidation chemical is hydrogen peroxide or carbamide peroxide or dioxirane or oxone.

4. A single stage bleaching procedure for teeth comprising the administration to teeth of:

- a formulation comprising the enzyme papain and activating co-ingredients or
- a formulation comprising the enzyme tannase or
- a formulation comprising dioxirane.

5. A composition for whitening teeth according to claim 1, 2 and 3 comprising:
- the enzyme tannase and/or
- the papain enzyme and activating ingredients and/or
-the oxidizing agent, dioxirane .

6. A composition for enhancing the whitening response of stain components to oxidative chemicals in a bleaching procedure according to claim 1 and 2, comprising the laccase enzyme and hydroxybenzotriazole.

7. A composition for enhancing the whitening response of stain components to oxidative chemicals according to claim 6, whereby the oxidative chemical is hydrogen peroxide or carbamide peroxide or dioxirane or oxone.

8. A composition according to claim 5, comprising the enzyme tannase and one or more additional enzymes.

9. A composition according to claim 8, whereby the additional enzyme can be an oxido-reductase, a laccase, an oxidase, lyzozyme, papain.

10. A composition according to claim 5, comprising the enzyme tannase and co-ingredients selected from the list: cysteine, cystine, EDTA, mercaptoethanol, dithiothreitol, metabisulfite salts, N-bromosuccinimide, hydrogenperoxide and carbamide peroxide.

11. A composition according to claim 5, comprising the enzyme tannase, cysteine, EDTA and mercaptoethanol.

12. A composition according to claim 5, comprising the enzyme tannase derived from Aspergillus Oryzae.

13. A composition according to claim 8,9,10,11,12 comprising the enzyme tannase, for use at a temperature between 0C and 80C (preferably 35 to 45 C) and a pH range between 2 and 9 (preferably between 4 and 6).

14. A composition according to claim 5, comprising the enzyme papain and one or more of the following co-ingredients: cysteine, mercaptoethanol, dithiothreitol, EDTA, metabisulfite salts,N-bromosuccinimide and cystine.

15. A composition according to claim 14, comprising the enzyme papain, cysteine, EDTA and a thiol containing product.

16. A composition according to claim 15, comprising one of the following thiol containing products: mercaptoethanol and dithiothreitol.

17. A composition according to claim 5, comprising the papain enzyme derived from the plant Carica Papaya.

18. A composition according to claim 5, comprising the papain enzyme, and one or more additional enzymes.

19. A composition according to claim 18 whereby the additional enzyme can be tannase, lyzozyme, laccase and oxido-reductase.

20. A composition according to claim 14,15,16,17,18,19 comprising the papain enzyme for use at a temperature between 10 C and 80 C (preferably 35 to 45 C) and a pH range between 4 and 9 (preferably between 6 and 8).

21. A composition for enhancing the response of stain components to oxidative chemicals according to claim 6, comprising the enzyme laccase, hydroxybenzotriazole and one or more of the following enzymes: papain, tannase, lyzozyme and Mn-peroxidase.

22. A composition according to claim 6, whereby the laccase enzyme originates from one of the following sources: Collybia, Fomes, Lentinus, Pleurotus, Aspergillus, Neurospora, Podospora, Phlebia, Coriolus (eg. Hirsutus), Botrytis, Polyporus sp., M. thermophila, Rhizoctonia sp or Rhus sp.

23. A composition according to claim 6, comprising the enzyme laccase for use at a temperature between 10 C and 80 C (preferably 35 to 45 C) and a pH range between 4 and 9 (preferably between 4.5 and 7.5).

24. A composition according to claim 5, comprising the chemical dioxirane, whereby dioxirane has been prepared in situ from oxone and a keton catalyst.

25. A composition according to claim 24 whereby the keton catalyst is chosen from the group comprising: acetone, cyclohexanone, 1,4-cyclohexane-dione, cyclic ketones with more than one cyclic ring structure and bis(triethylphosphine)-platinum (II)-oxalate.

26. A bleaching procedure according to any of the claims 1 to 3, whereby the dioxirane is produced in situ at a temperature between 0C and 50 C and at a pH between 4 to 9, preferably 6 to 7.5.

27. A multi-stage- bleaching procedure according to claims 1 to 2, whereby at least one stage comprising an oxidizing agent is preceded by one or more treatments with one or more enzyme formulations according to claims 5 and 6.

28. A multi-stage-bleaching procedure according to claim 27 whereby the oxidizing agent is hydrogen peroxide, carbamide peroxide, dioxirane or oxone.

29. A multi-stage bleaching procedure whereby enzyme formulations according to claim 5 and 6 are re-used in alternating order in repeated treatment stages.

Fig. 1

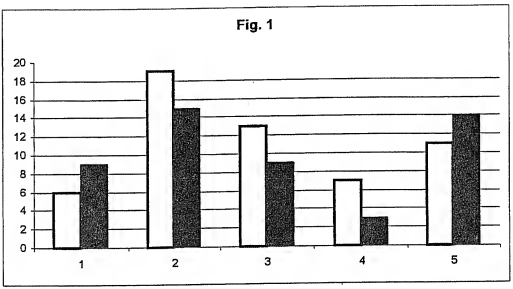


Fig. 2

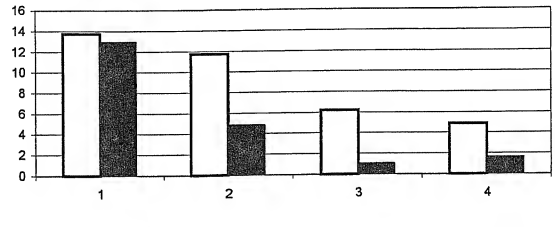


Fig. 3

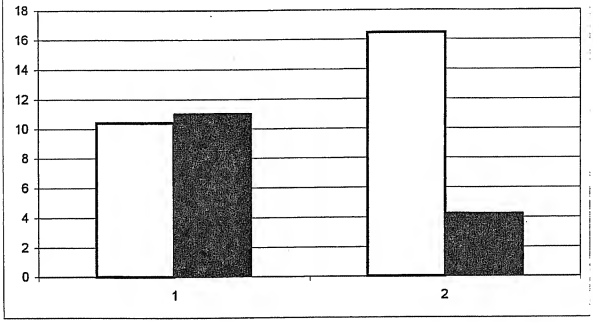
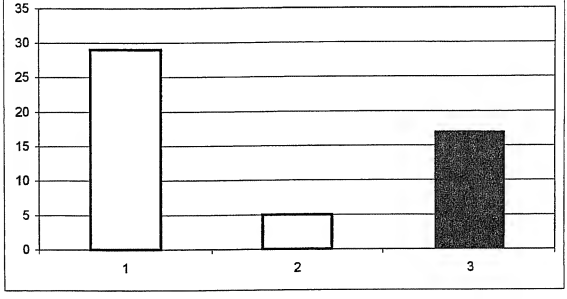


Fig. 4



INTERNATIONAL SEARCH REPORT

Inte .onal Application No
PCT/EP 00/10698

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K7/28 A61K7/20 A61K7/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FR 2 345 998 A (MANCEAU LABORATOIRES) 28 October 1977 (1977-10-28) page 2, line 17 - line 20 page 3, line 5 - line 13 claims ---	1,4,5, 14-16
A	WO 97 06775 A (NOVONORDISK AS) 27 February 1997 (1997-02-27) cited in the application page 9, line 29 - line 34 claims 1,3,4,15-17,19,20 ---	1,6,22, 23
A	DE 19 44 904 A (WOLF UVE) 1 April 1971 (1971-04-01) the whole document ---	1,4,5,8, 9
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 March 2001	02/04/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Pelli Wablat, B

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INTERNATIONAL SEARCH REPORT

Inte .onal Application No
PCT/EP 00/10698

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	GB 2 304 107 A (CIBA GEIGY AG) 12 March 1997 (1997-03-12) page 3, paragraph 3 page 15, last paragraph page 16	1,2,6, 21-23
A	WO 97 11676 A (MONTGOMERY ROBERT ERIC) 3 April 1997 (1997-04-03) page 5, line 18 - line 25 claims page 6, line 20 - line 26 page 5, line 29	1-3,27, 28

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter ional Application No
PCT/EP 00/10698

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